

Applicants: Iva Greenwald and Diane Levitan
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Filed: March 27, 1998
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Amendments to the Specification:

Please insert the following paragraph at page 8, line 3, of the specification:

The plasmids designated pMX8 and p1-1E were deposited on September 14, 1995, pursuant to the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209, under ATCC Designation Nos. 97278, and 97279, respectively. During the pendency of the subject application, access to the deposit shall be afforded to the Commissioner upon request. All restrictions upon public access to this deposit shall be removed upon the grant of a patent on this application and the deposits shall be replaced if viable samples cannot be made by the depository named hereinabove.

Please replace the paragraph beginning at page 14, line 5, with the following amended paragraph:

Applicants genetically mapped *sel-12* to the left of *unc-1* X: from hermaphrodites of genotype *sel-12(ar131)* *dpy-3(e27)/unc-1(e538)*, 1/36 Sel non-Dpy and 18/19 Dpy non-Sel recombinants segregated *unc-1*. To clone *sel-12*, applicants used the well correlated genetic and physical maps in the *sel-12* region to identify cosmid clones that potentially carried the *sel-12* gene (ref. 27 and A. Coulson et al., personal communication). Applicants assayed pools and single cosmids for the ability to rescue the Egl defect of *sel-12 (ar131)* hermaphrodites, using the plasmid pRF4 [rol-6 (su1006)] as a dominant cotransformation marker (28). Ultimately, applicants found that pSpX4, containing a 3.5 kb *SpeI/+Xho I* subclone of C08A12 (Subcloned into KS Bluescript, Stratagene)

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completely rescue *sel-12(ar131)*. When this subclone was microinjected at a concentration of 10 µg/ml into *sel-12(ar131)* animals, 6/6 lines all demonstrated rescue of the Egl phenotype. When applicants attempted to obtain transgenic lines carrying pSpX4 using a concentration of 50 µg/ml, applicants obtained F1 transformants but no stable lines perhaps indicating some toxicity of this plasmid at higher concentrations. Applicants used this genomic subclone to screen a cDNA library (~~kindly provided by Bob Barstead~~) and identified one class of clones of 1.5 kb in size. All subcloning, restriction digests, and library screening were done according to standard techniques (29). Applicants sequenced both strands of the cDNA clone after generating systematic deletions using the Erase-a-base system (Promega®). DNA sequence was performed on double stranded templates using Sequenase™ (US Biochemical). The cDNA contained both a poly (A) tail and a portion of the spliced leader sequence SL1 (ref. 30), suggesting it was a full length clone. Applicants confirmed the 5' end of the cDNA by reverse transcription followed by polymerase chain reaction (RT-PCR) (31). The sequence of this full-length cDNA can be found through GenBank under accession number U35660.

Please replace the paragraph beginning at page 14, line 39, with the following amended paragraph:

To identify the lesions associated with *sel-12* alleles applicants used PCR to amplify the *sel-12* genomic fragment from DNA isolated from the *sel-12* mutant strains using the primers DL103 (5' TGTCTGAGTTACTAGTTTCC 3') (SEQ. ID. NO:7) and DLG3 (5' GGAATCTGAAGCACCTGTAAGCAT 3') (SEQ. ID. NO:8). An aliquot of this double-stranded amplification product was used as the template in a subsequent round of PCR using only the primer DL103, to generate a single-stranded template. Exon specific primers were used to determine

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the entire coding sequence for all three alleles. For each allele, only one alteration in sequence was identified.